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Deep sleep after social stress: NREM sleep slow-wave activity is enhanced in both winners and losers of a conflict

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ABSTRACT

Sleep is considered to be a recovery process of prior wakefulness. Not only duration of the waking period affects sleep architecture and sleep EEG, the quality of wakefulness is also highly important. Studies in rats have shown that social defeat stress, in which experimental animals are attacked and defeated by a dominant conspecific, is followed by an acute increase in NREM sleep EEG slow wave activity (SWA). However, it is not known whether this effect is specific for the stress of social defeat or a result of the conflict per se. In the present experiment, we examined how sleep is affected in both the winners and losers of a social conflict. Sleep–wake patterns and sleep EEG were recorded in male wild-type Groningen rats that were subjected to 1 h of social conflict in the middle of the light phase. All animals were confronted with a conspecific of similar aggression level and the conflict took place in a neutral arena where both individuals had an equal chance to either win or lose the conflict. NREM sleep SWA was significantly increased after the social conflict compared to baseline values and a gentle stimulation control condition. REM sleep was significantly suppressed in the first hours after the conflict. Winners and losers did not differ significantly in NREM sleep time, NREM sleep SWA and REM sleep time immediately after the conflict. Losers tended to have slightly more NREM sleep later in the recovery period. This study shows that in rats a social conflict with an unpredictable outcome has quantitatively and qualitatively largely similar acute effects on subsequent sleep in winners and losers.

1. Introduction

Sleep is a complex phenomenon that consists of two distinct stages, non-rapid-eye-movement (NREM) sleep and rapid-eye-movement (REM) sleep. In general, NREM sleep is thought of as a process during which the brain recovers from prior wakefulness. This notion is supported by the finding that extended wakefulness is followed by a compensatory increase in both NREM sleep time and intensity (Benington and Heller, 1995). The NREM sleep intensity is reflected in the amount of slow waves in the electro-encepha-lo-gram (EEG) (Blake and Gerard, 1937; Friedman et al., 1978; Borbély and Neuhaus, 1979). EEG slow wave activity (SWA) at the start of NREM sleep, i.e. the spectral power in the 1–4 Hz range, is a function of the duration of prior wakefulness: the longer the period of wakefulness, the higher SWA is at the beginning of NREM sleep (Tobler and Borbély, 1986; Dijk et al., 1987; Franken et al., 1991; Lancel et al., 1992; Huber et al., 2000). Moreover, SWA is highest at the beginning of the sleep phase and gradually declines during the course of sleep as the NREM sleep debt dissipates (Tobler and Borbély, 1986; Dijk et al., 1987; Franken et al., 1991; Lancel et al., 1992; Huber et al., 2000). Yet, several studies have shown that EEG SWA during NREM sleep not only depends on the duration, but also on the quality of prior wakefulness. A number of studies have suggested that the experience of stress during wakefulness may accelerate the build up in sleep debt and increase the need for sleep (Meerlo et al., 1997, 2001a).

A social conflict in rodents is a natural stressor causing strong classical neuroendocrine and behavioral stress responses (Koolhaas et al., 1997a,b). Moreover, the experience of defeat in a social conflict is a rather dramatic event associated with behavioral and physiological changes that can last for several days up to weeks after the conflict (Meerlo et al., 1996a,b; Miczek et al., 1990). Social defeat has also been found to have immediate effects on subsequent sleep: rats (Meerlo et al., 1997, 2001a) and mice (Meerlo and Turek, 2001) showed increased amounts of NREM sleep and/or increased NREM intensity, as reflected in elevated EEG SWA. In the social defeat paradigm, experimental animals are placed in the territory of an older and larger conspecific. Under

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these conditions, the experimental animals generally exhibit little to no aggression and are subjected to the conflict with the cage owner without any control over this situation. Yet, it is not known whether the subsequent increases in NREM sleep time and/or SWA are due to the lack of control and the experience of defeat or due to the conflict per se. It may be that a social conflict with more opportunities to influence the outcome has different effects on subsequent sleep. In a conflict where subjects are matched to each other and are confronted in a neutral area, it is possible for both of them to come out as a winner or a loser. No studies have investigated the effect of such social confrontations on subsequent sleep.

For these reasons, the aim of this study was (1) to examine the effect of a social conflict with an unpredictable outcome on subsequent recovery sleep, and (2) to explore differential effects between winners and losers of the fight. A gentle stimulation control procedure was included to differentiate between effects of social conflict and effects due to sleep deprivation.

2. Material and methods

The study was performed with 6-month-old male wild-type Groningen (WTG) rats (*Rattus Norvegicus*), originally wild-trapped animals and bred under laboratory conditions for over 50 generations in the animal facility of the University of Groningen. The animals were individually housed under a 12-h light/12-h dark cycle with lights on from 10.00 to 22.00 h. Housing rooms had stable temperature (21 ± 1 °C) and humidity (60 ± 2%) and water and food were provided ad libitum throughout the experiment. Before the start of the experiment, the aggression levels of the experimental animals were characterized based on their attack latencies and amount of aggressive behavior during a standard resident-intruder test (see for procedure video publication by Koolhaas et al., 2013). In short, the animals were confronted in their home cage with a younger intruder on four consecutive days. On the first 3 days, these intruders were removed from the cage after the first attack by the resident. On the fourth day, aggressive behavior of the residents was recorded and analyzed for 10 min after the first attack. As common in this test, all residents attacked and defeated the intruder (Koolhaas et al., 2013).

2.1. Social conflict

During the experiment, the animals that had been selected from the breeding colony were subjected to a social conflict by confronting them with each other. Opponents were paired on the basis of similar aggression levels recorded in the resident-intruder tests prior to the experiments, making it impossible to predict a winner and loser in advance. The confrontations took place during the sixth hour of the light phase in a large neutral cage ($80 \times 55 \times 50$ cm) instead of the home cages in which sleep recordings were performed. Animals were habituated to this test cage for 3 days prior to the start of the baseline recordings in their home cage. Habituation was performed to reduce novelty stress and to promote aggression in the cage where the experimental conflict would take place. During this habituation period animals were kept apart from each other by a non-transparent divider, which separated the test cage in two different compartments. After 3 days animals were placed back into their home cage, habituated again to the cables and their home cage for approximately 18 h, after which the 24-h baseline recordings started. The next day, animals were taken from their home cage 15 min prior to the start of the social conflict and placed on their own side of the test cage. On the start of the sixth hour of the light phase, the non-transparent divider was taken away and the social interaction between the animals took place. Animals were identified as losers when in the course of the conflict they showed fleeing, freezing and submissive behavior. Winners were characterized by attacking with biting and clinching and inducing submissive behavior in their opponent. Animals from pairs were no clear conflict took place or conflicts with an uncertain outcome were excluded from the final analysis. Thus, only undoubted winners and losers were used. After a winner and a loser of the fight were identified a transparent divider was put in the cage for the remainder of the hour. This ensured that the social aspect of the interaction continued by visual confrontation, but prevented the animals from seriously harming each other.

2.2. Gentle stimulation

A 1-h sleep deprivation control procedure was included to differentiate effects from the social conflict from normal recovery after sleep loss. A gentle stimulation procedure was applied, which consisted of keeping the rats awake with as little disturbance as possible by tapping the cage and gently shaking the cage (see for procedure Meerlo and Turek, 2001; Van der Borght et al., 2006).

2.3. Sleep recordings

For sleep recordings, selected animals were implanted with permanent electrodes to record cortical EEG and neck electromyogram (EMG). Surgery was performed under Isoflurane (Pharmachemie BV, Harlem, The Netherlands) anesthesia. Three steel screws (diameter 1 mm) through the skull served as electrodes. One screw was placed above the right hemisphere, 1 mm anterior and 2.5 mm lateral from bregma. The second screw was placed above the left hemisphere 3 mm posterior and 2.5 mm lateral from bregma. The third screw, which functioned as ground electrode, was placed 4 mm posterior and 2 mm lateral from bregma above the right hemisphere. Two insulated stainless steel wires were inserted subcutaneously on the neck muscle to record the EMG. The electrodes were attached to a connector, which was cemented to the skull with dental acrylic. After at least 2 weeks of recovery, animals were habituated to handling and hooking up to the recording cable. The recording cable was attached to a swivel, which allowed free movement throughout the cage. After at least 2 days of habituation, EEG and EMG recordings started. Signals were fed to an amplifier (Vitaport 0212-56 TEMEC Instruments B.V.), which was connected to a computer with the recording program (Columbus™ Version 1.09.05, TEMEC Instruments B.V.) where data were collected and saved. This computer was located outside of the room where animals were placed, allowing checks and manipulations without disturbing the animals. The EEG signal was amplified 10.000 times, high-pass filtered at 1 Hz and low-pass filtered at 30 Hz. The EMG signal was amplified 5000 times, high-pass filtered at 1,5 Hz and low-pass filtered at 150 Hz. The signals were converted to digital format and stored at 128 Hz resolution. EEG and EMG were measured for 2 blocks of 2 consecutive days, consisting of a baseline day and the experimental day, starting at lights-on. During the sixth hour of the light phase on the experimental day animals were subjected to gentle stimulation in the first 2-day block and social conflict in the second 2-day block. The remaining 18 h of an experimental day (the second half of the light phase and the following dark phase) were considered the recovery period. A new baseline recording preceded each experimental procedure. There were two weeks in between the gentle stimulation and social conflict condition.

2.4. Analysis of vigilance states

By visual inspection of the EEG and EMG signals, 10-s epochs were classified as either wakefulness, NREM sleep or REM sleep

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(Meerlo and Turek, 2001), using a sleep processing program (Vitascore v 1.30, Temec Instruments). The EEG signal was subjected to spectral analysis by fast Fourier transformation and, for all NREM sleep epochs, the EEG power in the delta or slow wave range (1–4 Hz) was calculated. To correct for inter-individual differences in strength of the EEG signal, the delta power values were normalized for animals by expressing them relative to their own baseline delta power, i.e. the NREM sleep delta power values per time interval were expressed as a percentage of the average 24-h baseline NREM sleep delta power value. These normalized delta power values are referred to in the text and figures as slow wave activity (SWA). For winners and losers of the conflict, the accumulated NREM sleep SWA over the total 18-h recovery period was expressed as a percentage of the corresponding 18-h baseline NREM SWA and is referred to in the text as cumulative slow wave energy (SWE). For presentation and statistical analysis of the data, NREM and REM sleep time and NREM sleep SWA were calculated for 2-h intervals and for the total 18-h recovery period.

2.5. Statistics

Since the two baselines that preceded each of the experimental conditions did not differ from each other in sleep–wake pattern and EEG SWA, they were averaged for presentation in the figures and for statistical analysis. To test for acute effects of the experimental manipulations on sleep parameters, we analyzed the first 2-h interval following the experimental treatments and the corresponding 2-h baseline interval with a one-way ANOVA with factor condition (baseline, gentle stimulation, or social conflict). When ANOVA showed a significant condition effect, Tukey post-hoc tests were performed. Differences in sleep parameters across the 18-h recovery periods were further investigated by repeated measures ANOVA with a factor condition (baseline, gentle stimulation, or social conflict) and a factor time interval (nine consecutive 2-h blocks). When the overall repeated measures ANOVA revealed a significant effect of condition or a significant condition × time interaction, post hoc *t*-tests were applied to determine at which 2-h intervals the differences occurred. Finally, overall differences in the total amount of NREM and REM sleep during the 18-h recovery period were examined using a one way ANOVA with post hoc *t*-tests. All analyses were performed with the software IBM PASW Statistics (version 18 for Windows). Significance level was set to $p < 0.05$. Data shown in text and figures are averages ± standard error of the mean (SEM).

3. Results

3.1. Animals

Eighteen rats were selected from the breeding colony. For 6 animals (3 pairs) no clear winner or loser could be identified after the conflict and therefore, they were not included in the final analysis. Three of the remaining 12 animals were lost due to poor EEG-signals, leaving data from 9 animals (4 winners and 5 losers) to be analyzed. During the social conflict animals attacked/were attacked after 727 ± 327 s on average.

3.2. Social conflict versus gentle stimulation

3.2.1. NREM sleep

The amount of NREM sleep per 2-h interval is depicted in Fig. 1. The 1-h sleep deprivation by gentle stimulation or social conflict did not have an acute effect on the amount of NREM sleep in the first 2-h interval after the treatment (One-way ANOVA: $F_{2,24} = 0.07$, $p = 0.931$). In the course of the 18-h recovery period following the treatment, i.e., the remainder of the light phase and subsequent dark phase, the animals that had experienced a social conflict tended to sleep more than in the baseline condition and the gentle stimulation condition. Repeated measures ANOVA for the entire 18-h recovery period indicated a trend toward an effect of the condition ($F_{2,24} = 2.89$, $p = 0.075$). There was no condition × 2-h interval interaction ($F_{16,192} = 0.87$, $p = 0.559$).

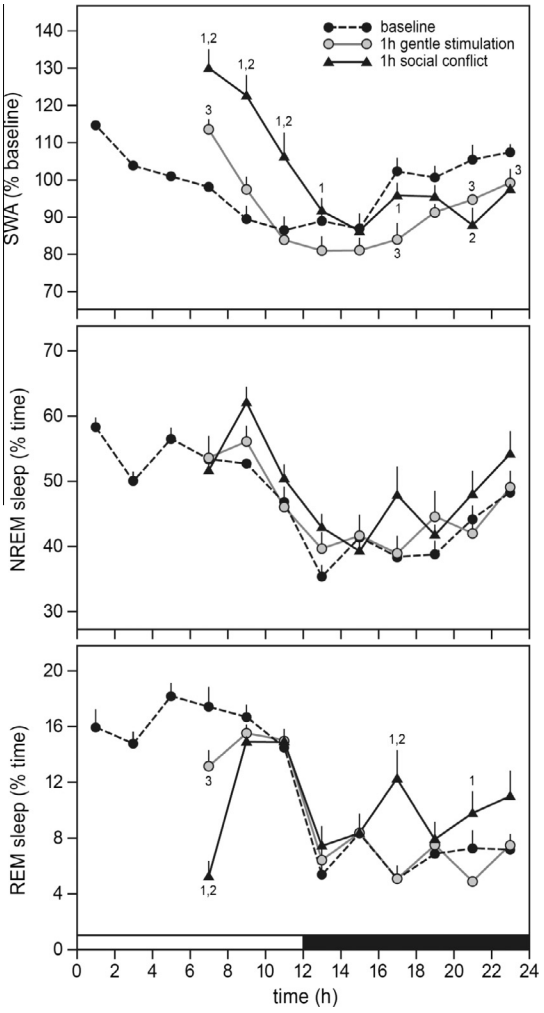

Fig. 1. Two-hourly values of REM sleep, NREM sleep and NREM sleep SWA in male WTG rats subjected to 1-h of gentle stimulation or 1-h of social conflict with a matched conspecific ($N = 9$ for each condition). Experimental manipulations took place in the middle of the light phase. Only when repeated measures ANOVA revealed a significant effect of condition effect or a condition × 2-h interaction effect, were successive 2-h intervals compared using *t*-tests. Significant differences: (1) between social conflict and gentle stimulation, (2) between social conflict and baseline, (3) between gentle stimulation and baseline (two-tailed paired *t*-test, $p < 0.05$).

The total NREM sleep time during the 18-h recovery period after the conflict (8 h 49 min ± 17 min) was significantly higher than during the same period of the baseline condition (7 h 59 min ± 10 min; $t(16) = 2.57$, $p < 0.05$) but not significantly different from the total NREM sleep time after sleep deprivation by gentle stimulation (8 h 14 min ± 18 min; $t(16) = 1.44$, $p = 0.169$). Total NREM sleep time during the 18-h recovery period after gentle stimulation did not significantly differ from baseline ($t(16) = 0.76$, $p = 0.458$).

3.2.2. NREM sleep SWA

As expected, the NREM sleep that was lost during the experimental treatments induced a compensatory increase in subsequent

NREM sleep SWA but this increase in SWA was higher after the social conflict than after gentle stimulation (Fig. 1). There was an overall significant effect of condition for the first 2 h interval after the manipulation (One-way ANOVA: $F_{2,23} = 26.35$, $p < 0.001$). Compared to the corresponding baseline levels, SWA was significantly elevated after both gentle stimulation ($p < 0.01$) and social conflict ($p < 0.001$). SWA after the conflict in turn was higher than SWA after gentle stimulation ($p < 0.01$).

Repeated measures ANOVA for the entire 18-h recovery period revealed a significant condition effect ($F_{2,23} = 8.50$, $p < 0.01$) and a significant condition × 2-h interval interaction ($F_{16,184} = 7.51$, $p < 0.001$). Tukey post hoc analyses showed a trend in the difference from social conflict to baseline ($p = 0.064$). Gentle stimulation did not differ significantly from baseline ($p = 0.196$), but did from social conflict ($p < 0.001$). The increase in SWA after a social conflict persisted during the remainder of the light phase, while SWA following gentle stimulation normalized already after the first 2 h period (Fig. 1). Moreover, SWA levels dropped significantly below baseline levels in the late dark phase after gentle stimulation, but to a lesser extent after social conflict (see Fig. 1 for details).

3.2.3. REM sleep

The detailed pattern of REM sleep per 2-h intervals is shown in Fig. 1. One-way ANOVA revealed a significant acute treatment effect on the amount of REM sleep in the first subsequent 2-h interval ($F_{2,24} = 25.00$, $p < 0.001$). After both gentle stimulation and social conflict, the amount of REM sleep during the first 2-h interval was lower than the amount during corresponding baseline interval ($p < 0.05$ and $p < 0.001$, respectively). This acute decrease in REM sleep was stronger after an hour of social conflict than after an hour of gentle stimulation ($p < 0.001$).

Repeated measures ANOVA for the entire 18-h recovery period showed no significant overall condition effect ($F_{2,24} = 1.07$, $p = 0.359$) but did reveal a significant condition × 2-h interval interaction ($F_{10,116} = 5.13$, $p < 0.001$). The acute suppression of REM sleep during the first 2-h interval following the social conflict was partly compensated for by higher amounts of REM sleep in the subsequent dark phase (see Fig. 1 for details).

Overall, the total REM sleep time in the 18-h recovery period after the experimental manipulations did not differ significantly from each other and from baseline (One-way ANOVA: $F_{2,24} = 1.09$, $p = 0.353$). On average, animals spent 1 h 52 min ± 8 min in REM sleep during the recovery period after the conflict, which is not different from 1 h 40 min ± 4 min after gentle stimulation and 1 h 47 min ± 5 min during the corresponding baseline period.

3.3. Winners versus losers

3.3.1. NREM sleep

The time course of NREM sleep in animals that won the conflict and animals that lost the conflict is shown in Fig. 2. There was no difference between winners and losers in the acute effect of the conflict on the amount of NREM sleep (*t*-test for the first 2 h interval: $t(7) = 0.96$, $p = 0.370$). Interestingly, for the entire 18-h recovery period after the conflict, repeated measures ANOVA showed a nearly significant condition effect ($F_{1,7} = 5.11$, $p = 0.058$) but no condition × 2-h interval interaction ($F_{8,56} = 0.57$, $p = 0.798$). Overall, there was a clear trend for more NREM sleep in the animals that lost the conflict as compared to the winners.

The total amount of NREM sleep for the entire 18-h recovery period following the interaction seemed to be higher in animals who had lost the fight (9 h 17 min ± 13 min) compared to the winners (8 h 14 min ± 27 min; $t(7) = 2.30$, $p = 0.055$).

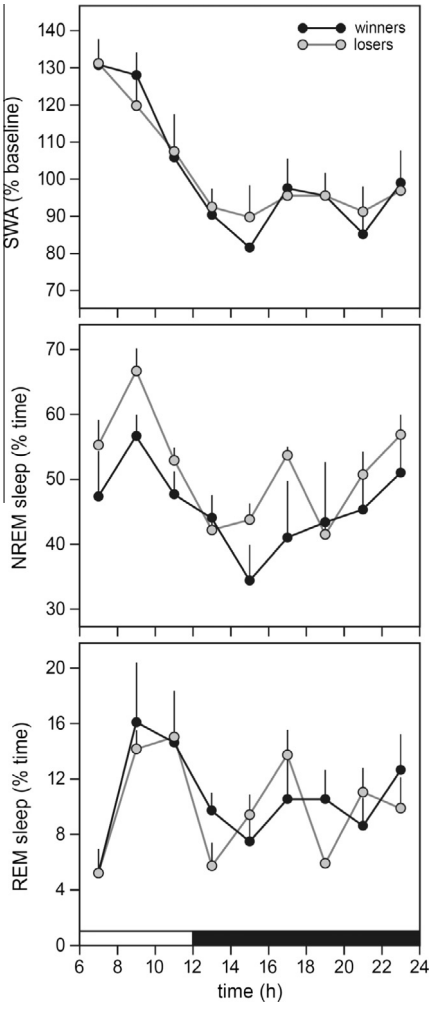

Fig. 2. Two-hourly values of REM sleep, NREM sleep and NREM sleep SWA in male WTG rats subjected to 1-h of social conflict, split into animals who won (winners, $N = 4$) or lost (losers, $N = 5$) the fight. The social conflict took place in the middle of the light phase. Repeated measures ANOVA revealed no significant condition effect or condition × 2-h interaction effects. However, losers tended to have more NREM sleep during the recovery period after the conflict compared to winners (repeated measures ANOVA showed a trend for the condition effect ($F_{1,7} = 5.11$, $p = 0.058$)).

3.3.2. NREM sleep SWA and SWE

The acute increase in NREM sleep SWA seen in the first 2-h interval after the conflict was similar in the winners and losers of the fight (Fig. 2). Also the SWA pattern across the entire recovery period did not significantly differ between winners and losers (repeated measures ANOVA: condition effect $F_{1,6} = 0.02$, $p = 0.887$ and condition × 2-h interval interaction $F_{8,48} = 0.32$, $p = 0.956$). The accumulated SWE after the recovery period was higher in losers (121 ± 8%) than in winners (111 ± 6%), but the level of statistical significance was not reached ($t(7) = 2.07$, $p = 0.077$).

3.3.3. REM sleep

The acute suppression of REM-sleep immediately after the conflict was comparable for winners and losers ($t(7) = 0.09$, $p = 0.929$; see Fig. 2). The compensation for this acute drop in REM sleep developed in the same manner for those animals that had lost and won the fight. Repeated measures ANOVA for the entire 18-h recovery period revealed no condition effect ($F_{1,7} = 0.16$, $p = 0.703$) and no condition × 2-h interval interaction ($F_{8,56} = 0.89$, $p = 0.528$). In line with this, the total amount of REM sleep in the 18-h recovery period did not differ between winners

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and losers (1 h 56 min ± 12 min and 1 h 57 min ± 11 min, respectively; $t(7) = 0.40$, $p = 0.701$).

4. Discussion

We confirmed that in rats the experience of a social conflict strongly increases EEG slow-wave activity during subsequent sleep. This increase was not explained by sleep loss per se, as the increase in SWA was significantly higher than in animals sleep deprived for the same duration by gentle stimulation. Since SWA is a generally accepted measure of sleep debt and sleep intensity (Tobler and Borbély, 1986; Dijk et al., 1987; Franken et al., 1991; Lancel et al., 1992; Huber et al., 2000), our study once again confirms that the build-up of sleep debt not only depends on the duration of prior wakefulness but also on the nature of that wakefulness (Meerlo et al., 1997, 2001a). A social conflict may represent an intense form of wakefulness that requires more intense recovery sleep.

Importantly, the present study showed that the initial increase in NREM sleep SWA was not different between animals that had won and animals that had lost the conflict, which indicates that the increase was caused by the conflict per se and not the outcome. Also, SWA normalized gradually after the conflict, without showing any pattern differences between winners and losers. Given that sleep is generally considered to be important for recovery and plasticity of the brain, the acute increase in NREM sleep SWA may be an adaptive response to the increased overall or local brain activity during the conflict, shared by both winners and losers.

While the losers and winners of a conflict showed similar acute increases in NREM sleep SWA, losers showed a tendency for a mild increase in NREM sleep compared to winners, although it did not reach statistical significance because of the limited sample size ($p = 0.055$). This tendency for a delayed increase in NREM sleep in our losers may reflect a slightly increased need for recovery sleep. The potential mechanism underlying such an increase in NREM sleep time remains unknown but somewhat parallels physiological and endocrine responses seen in losers. While losers and winners of a conflict were reported to have a fairly similar peak response in blood pressure, heart rate and corticosterone, the elevations of these measures persisted longer in the losers (summarized in Figs. 3 and 4 of Koolhaas et al., 2011). It may be that similar acute increases in NREM sleep SWA in winners and losers are related to the similar peak values of the physiological responses whereas the tendency for an increase in NREM sleep time in the losers is related to the slower recovery of the physiological responses in these animals. On the other hand, most published data on different physiological responses between winners and losers are based on the classical resident-intruder paradigm, with the resident being the territorial cage owner, aggressor and almost certain winner of the conflict whereas the socially inexperienced and non-aggressive intruders are the almost certain losers of the conflict (Koolhaas et al., 2013). This is slightly different from the paradigm used in the current experiment, where two animals that were matched in age and aggression were confronted with each other in a test cage equally familiar to both of them, which made the outcome of the conflict unpredictable. Therefore, the detailed time course of the physiological and endocrine responses may be different in the present experimental setup, compared to winners and losers in the classical resident-intruder paradigm. Speculatively, our winners and losers may have shown less outspoken differences in physiological responses as both animals to some degree experienced uncontrollable social stress.

In contrast to NREM sleep, REM sleep was strongly suppressed in the first couple of hours after the conflict in both winners and losers. The initial suppression of REM sleep may have been partly a consequence of the strong drive for NREM sleep as reflected in the high NREM sleep SWA. The animals compensated for the loss of REM sleep during and after the conflict, by having more REM sleep later on in the recovery phase. The acute suppression in REM sleep and compensation in the recovery phase is in agreement with what is found in other studies investigating the effects of social conflict (Meerlo et al., 1997, 2001a; Meerlo and Turek, 2001; Lancel et al., 2003). Interestingly, animals exposed to uncontrollable and inescapable foot shock stress were found to show significant reductions in REM sleep that were not recovered (Sanford et al., 2010) whereas controllable shocks were followed by an overall increase in REM sleep. Also restraint or immobilization stress was found to cause an overall increase in REM sleep beyond baseline levels (Rampin et al., 1991; Meerlo et al., 2001b). It has been suggested that such increases in REM sleep reflect an adaptive coping response that may serve the purpose of recovery (Sanford et al., 2010). However, it remains unclear why some stressors such as restraint are followed by an increase in REM sleep whereas others such as a social conflict promote NREM sleep. This complex variation in the effects of different stressors on sleep may depend on the nature, predictability and controllability of stressors, and their specific effects on physiology and brain function.

Importantly, while in humans stress is generally considered to be a major cause of disrupted sleep, our study confirms that in most animal models of acute stress, the arousing and sleep-inhibiting effects of stressors are rapidly overcome and are sometimes followed by increased sleep during the recovery phase (for review see Sanford et al., 2014). A possible explanation for this apparent difference is that in laboratory rodents the physiological activation and arousal rapidly disappear upon termination of the stressor and return to the home cage whereas human beings may carry their problems with them. Particularly in humans, stress is not necessarily always associated with an acute challenge but may be a cognitive and emotional phenomenon based on memories of past events as well as worries and expectations about the future. Humans may thus be capable of, more so than other animals, turning a single stressor or life event that occurred in the past, or even one pending in the future, into a persistent and chronic stress state (Sanford et al., 2014).

5. Conclusions

This study shows that the experience of a social conflict significantly increased NREM sleep SWA in rats, independent of the outcome of the conflict. This increase was caused by the conflict itself, and not the duration of wakefulness as confirmed by the significantly smaller increase in the gentle stimulation condition. Winners and losers did not differ in NREM sleep time, NREM sleep SWA and REM sleep time immediately after the conflict. Losers tended to sleep slightly more in the NREM sleep state in the prolonged recovery period. Whether or not this reflects a marginally greater recovery need remains uncertain.

6. Conflicts of interest and source of funding

Non declared.

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